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Association between Maternal Dietary Exposure to Food Chemicals Mixture during Pregnancy and Child Neurodevelopment: A Prospective Study

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List of acronyms

Acronym	Definition
AD	Alzheimer's disease
ADHD	Attention Deficit Hyperactivity Disorder
Al	Aluminum
ANSES	Agence nationale de la sécurité sociale
ASD	Autism Spectrum Disorder
Asi	Inorganic Arsenic
BFRs	Brominated Flame Retardants
BKMR	Bayesian Kernel Machine Regression
bw	Body Weight
Cd	Cadmium
CDI	Child Development Inventory
CI	Confidence Interval
Co	Cobalt
CondPIP	Conditional Posterior Inclusion Probability
CRESS	Centre de Recherche en Epidémiologie et Statistiques
CrVI	Chromium (VI)
DAG	Directed Acyclic Graph
DOHaD	Developmental Origins of Health And Disease
EAROH	Recherche sur les déterminants précoces de la santé
EFSA	The European Food Safety Authority
ELFE	Étude Longitudinale Française depuis l'Enfance
EPDS	Edinburgh Postnatal Depression Scale
FFQ	Food Frequency Questionnaire
Ge	Germanium
GroupPIP	Group Posterior Inclusion Probability
HAPs	Hazardous Air Pollutants
HgI	Inorganic Mercury
INRAE	Institut national de recherche pour l'agriculture, l'alimentation et l'environnement
IQ	Intelligence Quotient
IQR	Interquartile Range
LASSO	Least Absolute Shrinkage And Selection Operator
LB	Lower Bound
Li	Lithium
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit Of Detection
LOQ	Limit Of Quantification
Mn	Manganese
Ni	Nickel
PANDiet	Probability Of Adequate Nutrient Intake-Based Diet Quality Index
Pb	Lead
PCA	Principal Component Analysis
PCBs	Polychlorinated Biphenyls
PD	Parkinson's Disease
PFAAs	Perfluoroalkyl Acids
PIP	Posterior Inclusion Probability
PIQ	Performance Intelligence Quotient
PTWI	Provisional Tolerable Weekly Intake
PUFAs	Polyunsaturated Fatty Acids
QGC	Quantile G-Computation
Sb	Antimony
Sn	Tin
TDI	Tolerable Daily Intake
TDS2	The Second Total Diet Study
TWI	Tolerable Weekly Intake
UL	Tolerable Upper Intake Level
V	Vanadium
WHO	World Health Organization
WQS	Weighted Quantile Sum

Abstract

Background

Early life is a critical period for neurodevelopment, during which the nervous system is highly vulnerable to environmental factors. Maternal exposure to chemicals during pregnancy can transfer through the placenta to the fetal circulation. Numerous substances have been documented to have confirmed or potential neurotoxicity. Food is one of the primary pathways for exposure to these chemicals. The objective of the present study is to investigate the associations between maternal dietary exposure to food chemicals during the last three months of pregnancy and child neurodevelopment.

Methods

A total of 10,080 mother-child dyads from Étude Longitudinale Française depuis l'Enfance (ELFE) study were enrolled in the present study. Maternal dietary exposure to food chemicals was assessed by Food Frequency Questionnaire (FFQ). Child neurodevelopment was evaluated by the Child Development Inventory (CDI) score at 3.5 years old. After screening 210 chemicals, we focused our analysis on 14 metals as a mixture. We performed several statistical methods to analyze the effects of these chemicals. Principal component analysis (PCA) was used for dimensionality reduction and applied in multivariable linear regression. Bayesian kernel machine regression (BKMR) and quantile g-computation (QGC) were used to assess multiple exposures to mixtures.

Results

We obtained consistent results across all four statistical methods. The overall effect of the metal mixture was positively associated with child neurodevelopment at 3.5 years of age. Specifically, Antimony exhibited a negative association, whereas inorganic Mercury and Tin showed positive associations with child neurodevelopment. Additionally, Manganese and Germanium demonstrated positive associations in linear regression. No associations were found with other metals.

Conclusion

The present study showed various associations between maternal dietary exposure to metals during pregnancy and the child neurodevelopment.

Introduction

According to the developmental origins of health and disease (DOHaD) theory, prenatal and perinatal exposure to environmental factors plays a crucial role in the long-term development of children [1]. The fetal period is a critical phase during which the nervous system forms, and the developing brain is especially susceptible and vulnerable to environmental factors compared to the adult brain. [2–4]. Certain substances can cross the blood-placenta barrier from the mother to enter the child's circulation resulting in impairment, such as methylmercury [5] and fluoride [6].

Neurotoxicity is defined as the direct or indirect impact of chemicals that impair the nervous system in humans or animals [7]. A wide variety of substances have been reported to exhibit neurotoxic effects on humans, such as polychlorinated biphenyls (PCBs), arsenic, acrylamide, organophosphate pesticides, and hazardous air pollutants [8–10]. The susceptibility of the mammalian nervous system to chemical perturbations arises from its functional design features, given that nerve cells are prone to chemical attack at multiple loci [7]. Consequently, neurological dysfunction is one of the most common toxic responses to chemical substances among humans [7]. Neurotoxicity-induced diseases include Alzheimer's disease (AD), Parkinson's disease (PD), attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), intellectual disability and other cognitive impairments [2,8].

Epidemiological studies have shown the associations between prenatal exposure to certain substances and child neurodevelopment outcomes. For example, higher levels of prenatal exposure to phthalate metabolites and organophosphate esters, measured by urinary concentrations, have been discovered to be associated with poorer cognitive and behavioral outcomes in children aged 0–12 years [11,12]; perinatal exposure to PCBs could be associated with adverse cognitive development and attention in middle childhood [13]. However, results from previous studies have shown inconsistencies in various aspects. For instance, prenatal exposure to different types of PFAs yielded opposite results on the Performance Intelligence Quotient (PIQ) among children of different sexes aged 4-8 years [14] ; A cross-sectional study in Norway found that maternal blood concentrations of arsenic, cadmium and manganese in the 17th week of gestation were positively associated with the risk of autism spectrum disorder, while cesium, copper, mercury and zinc were negatively associated [15]. Furthermore, most previous epidemiological studies on environmental risk factors have mainly focused on the health effects of exposure to single substances, while real-world exposures typically involve complex mixtures of multiple chemicals [16,17]. Chemical mixtures have been increasingly studied, benefiting from emerging methods such as Bayesian Kernel Machine Regression

(BKMR), Weighted Quantile Sum (WQS), and Least Absolute Shrinkage and Selection Operator (LASSO). These models are designed to account for nonlinear relationships and multicollinearity of mixtures, which makes them more reliable and adaptable than other statistical models to screen out the dominant contributors to the joint effect. [18].

Diet is a major source of intake for numerous environmentally harmful substances [19]. Food can become contaminated during production or processing, or due to leakage from its packaging [20–26]. Longitudinal studies investigating the long-term neurodevelopmental outcomes in children due to prenatal dietary exposure to the mixture of neurotoxic substances have not yet been conducted. The present study aimed to utilize multiple statistical methods to investigate the associations between maternal dietary exposure to the chemical mixture during the last three months of pregnancy and child neurodevelopment.

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Methods

2.1 Study population

The present study was based on the prospective population-based cohort study "Étude Longitudinale Française depuis l'Enfance" (ELFE). ELFE study is the first French nationwide birth cohort study launched in 2011. This study recruited 18,329 newborns from 320 randomly selected maternity units in metropolitan France, including 289 pairs of twins. Recruitment was conducted during 25 selected days and grouped into four waves over the year [27]. The inclusion criteria were single or twin live births at 33 weeks of gestation or later, mothers aged 18 years or older, and no intention to leave metropolitan France within three years. Follow-up in the first 5 years mainly consisted of phone interviews, face-to-face interviews and self-reported questionnaires conducted with the parents.

2.2 Prenatal exposure to food chemicals

Maternal dietary intake

The assessment of maternal dietary exposure to chemicals during the last three months of pregnancy has been previously described [26]. During maternity stay, mothers completed a self-administered food frequency questionnaire (FFQ) to collect their dietary habits during the last three months, including the consumption of 125 food items, 12 non-alcoholic and 4 alcoholic beverages [28]. Portion size was based on photos from the SU.VI.MAX portion book [29] for 75 commonly eaten food items, and the midportion in the SU.VI.MAX portion book was automatically assigned for the 50 remaining food items. Following the transformation of FFQ frequency categories into daily frequencies, the daily intake of each food item was determined by combining the intake frequency and the portion size [28].

Maternal daily energy intake (kcal/day) was obtained by multiplying daily food intakes with the corresponding nutritional values in the SU.VI.MAX food composition database [30]. Maternal diet quality during the last three months was evaluated by the Probability of Adequate Nutrient intake-based Diet quality index score (PANDiet score), which ranges from 0 to 100 points, with higher scores indicating better nutritional adequacy [31].

Chemical content of food products

The second Total Diet Study (TDS 2) was undertaken by Agence nationale de la sécurité sociale (ANSES) and published in 2011. TDS 2 aimed to describe the French population's food-based exposure to substances of public health interest and to characterize the health risks linked to food and associated with these substances [32]. TDS 2 analyzed 445 substances in approximately 20,000 food products collected throughout metropolitan France, representing 90% of the diets of both adults and children in France [33,34]. For substances with concentrations below the limit of detection (LOD) or between the LOD and the limit of quantification (LOQ), the Lower Bound (LB) scenario was used, as recommended in GEMS/Food-EURO (2013) [35]. This scenario implies that non-detected values were replaced with 0 and values between the LOD and the LOQ were replaced by the values of LOD.

Estimation of maternal dietary exposure to chemicals

The women’s dietary intake in the ELFE study has been previously combined with the chemical content of food items in the TDS 2 [26]. For 210 substances in the ELFE study, the dietary exposure was above 0 for at least one woman.

2.3 Selection of chemicals

A mixture is defined as containing a minimum of three independent chemicals or chemical groups [16]. Mixture data is often high-dimensional and exhibits non-linear relationships with outcomes, which can render traditional statistical methods unsuitable or yield less robust results [17,36]. Moreover, the multicollinearity and interactions among mixture components — such as synergistic, antagonistic, and additive effects — add complexity and challenges to statistical methods [16,18]. It was not feasible to include all 210 substances in the analysis; therefore, we conducted the following process to select substances for the present study.

Initially, our analysis focused on chemicals with evidence of neurotoxic effect or biological plausibility of affecting neurodevelopment. We retrieved the conclusions of agency reports, literature reviews and articles published between 2014 and 2024 regarding the effects of all 210 substances on child neurodevelopment, cognitive and behavioral outcomes, as well as evidence from animal experiments. Epidemiological, toxicological and mechanistic evidences were considered simultaneously [37]. Chemicals demonstrating well-documented neurotoxic effects or neurotoxicity were classified as confirmed neurotoxins, while those with inconsistent or inconclusive findings were classified as potential neurotoxins. This step resulted in the exclusion of 63 chemicals lacking sufficient evidence of neurotoxic effects or neurotoxicity.

Next, Spearman correlations were conducted between each chemical and each food item, using 0.8 as an arbitrary correlation coefficient threshold. 52 chemicals strongly correlated ($r>0.8$) with at least one food item were excluded because high correlation between chemical and food item indicated overlapping intake patterns, making it difficult to distinguish the effects of the chemical from those of the food itself.

After these first two steps of selection, the remaining 95 food chemicals consisted of the following: 9 brominated flame retardants (BFRs), 8 dioxins, 19 hazardous air pollutants (HAPs), 14 metals, 17 polychlorinated biphenyls (PCBs), 22 pesticides, 3 perfluoroalkyl acids (PFAAs), acrylamide, bisphenol A, and fumonisin B1. In the subsequent study, we aimed to investigate the mixture effects of a chemical family.

Acrylamide, bisphenol A, and fumonisin B1 were excluded as they were the only representative of their chemical family. PFAAs, consisting of only three chemicals, were considered insufficiently complex for meaningful mixture analysis. Pesticides, PCBs and dioxins were not prioritized as their neurotoxic effects have been extensively studied [12,13,38]. HAPs were excluded due to their large number of compositions, which could potentially decrease model robustness and increase computation time and model complexity. Although animal experiments suggest potential neurotoxicity of BFRs [39,40], the limited and unclear mechanisms in existing evidence would make it difficult to compare and discuss our results with the literature.

After considering all the above-mentioned factors and excluding other chemical families, we ultimately focused the present study on 14 metals: aluminum (Al), inorganic arsenic (Asi), cadmium (Cd), inorganic mercury (HgI), manganese (Mn), lead (Pb), cobalt (Co), chromium (VI) (CrVI), germanium (Ge), lithium (Li), nickel (Ni), antimony (Sb), tin (Sn) and vanadium (V).

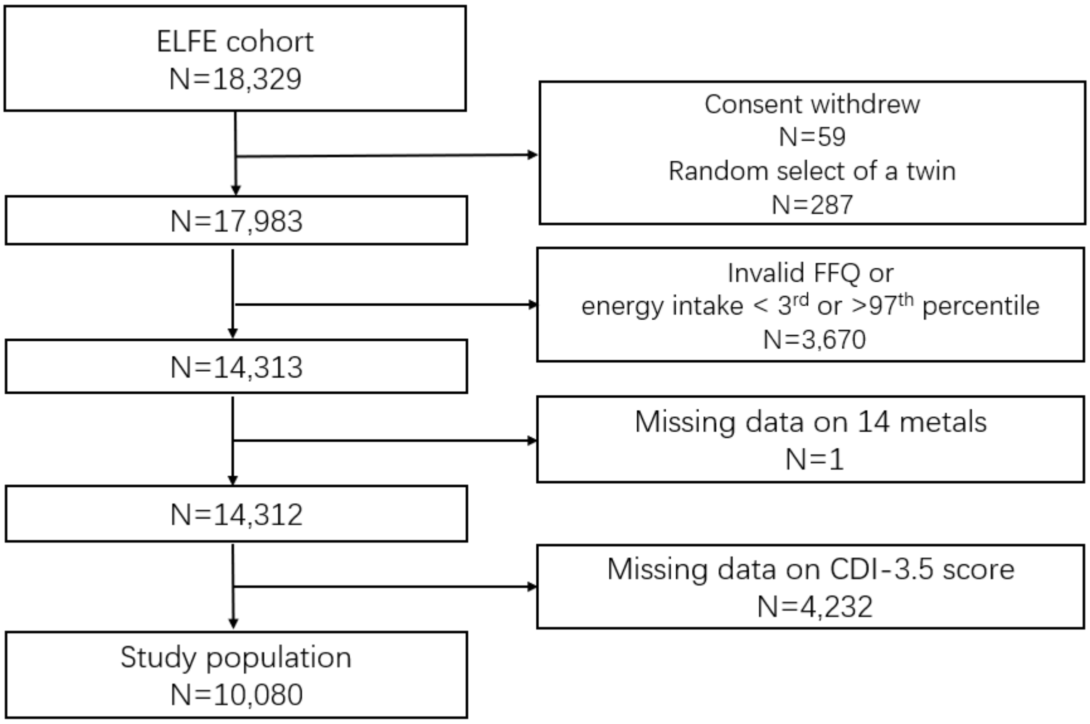
2.4 Assessment of child neurodevelopment

For this project, we decided to focus on child neurodevelopment assessed at 3.5 years. Parents complete an adapted version of the Child Development Inventory (CDI-3.5) by phone interviews [41,42]. CDI-3.5 included 8 domains of development: social, self-help, gross motor skills, fine motor skills, expressive language, language comprehension, characters and numbers. Each item is scored with 1 point if the child has acquired the ability and 0 if the ability has not been acquired. An overall CDI-3.5 score was calculated by summing the scores for all items, ranging from 17 to 62. A higher CDI-3.5 score indicates better neurodevelopmental attainment.

2.5 Selection of study sample

We excluded 59 participants who withdrew consent during the survey and randomly selected one infant from each of the 287 pairs of twins. Then we excluded participants without valid data on the FFQ or those who likely misreported their food intake (with a daily energy intake lower than the 3rd percentile, 933 kcal or higher than the 97th percentile, 5073 kcal) [43]. Next, we excluded participants with missing data in metals exposure or CDI-3.5 score. Finally, a total of 10,080 mother-child dyads were included in our study (Fig. 1).

Fig.1 Flowchart of the selection of study sample



2.6 Covariates

Maternal socio-economic and health characteristics were collected during the face-to-face interview at inclusion, the 2-month phone interview and maternal medical record. These covariates included maternal age at delivery (years), maternal pre-pregnancy body mass index (kg/m^2), maternal educational level (upper secondary or lower, intermediate, 3-y university degree, ≥5-y university degree), employment status during pregnancy (employed, unemployed, out of the labor force), monthly family income per consumption unit (€), Edinburgh Postnatal Depression Scale (EPDS) score [44], migration status (migrant/not born to French parents, descendant of at least one migrant parent, majority population/born to French parents), number of older children in the household (no sibling, 1 sibling, ≥2 siblings), maternal smoking during pregnancy (never smoker, smoker only before pregnancy, smoker only in early pregnancy, smoker throughout pregnancy). Region of residence (Paris region, North, East, Paris Basin – East, Paris Basin – West, West, Southwest, Southeast, Mediterranean) was derived from zip code.

2.7 Statistical methods

In all statistical models, metal concentrations have been log-transformed due to non-normal distribution. Following this transformation, all metal concentrations exhibited normal distributions. The MissForest imputation was performed to handle missing values in covariates [45]. We performed comparisons of continuous or categorical variables between the included and excluded populations at baseline using Student's t-test or chi-square test, respectively. Covariates used in the main analyses were identified from the literature and selected using the directed acyclic graph [46] (Fig. S1). All statistical models were adjusted for variables presented before: maternal age at delivery, maternal pre-pregnancy body mass index, maternal educational level, employment status during pregnancy, monthly family income per consumption unit, Edinburgh Postnatal Depression Scale score, migration status, number of older children in the household, maternal smoking during pregnancy, maternal daily energy intake, mother’s diet quality and region of residence, as well as variables related to study design (recruitment wave and maternity size) and child’s age at neurodevelopment assessment.

Firstly, as a preliminary and traditional approach, we performed multivariable linear regression to analyze the association of 14 metals and neurodevelopment at 3.5 years, considering all 14 metals simultaneously and adjusting for all covariates.

The following methods were employed to analyze the metal mixture, and their results were compared.

- Principal component analysis (PCA) was conducted to identify the patterns of maternal dietary exposure to chemicals. The identified principal components were included in a multivariable linear regression model to examine their effects on the child neurodevelopment outcomes, instead of 14 individual metals.

- The Bayesian Kernel Machine Regression (BKMR) model was used to examine the overall association of the mixture and the individual associations of 14 metals, as well as the interactions among the 14 metals. BKMR is developed to achieve variable selection, flexible estimation of the exposure-response relationship, and inference on the strength of the association between individual substances and health outcomes in a health effects analysis of mixtures simultaneously [17,47–49]. Furthermore, it can identify non-linear and non-additive relationships among chemicals in the mixture, and allows for adjustment for other variables. The group conditional posterior inclusion probabilities (groupPIPs) and conditional posterior inclusion probabilities (condPIPs) for individual metals in the mixture were obtained from the BKMR model with a hierarchical variable selection procedure (10,000 iterations by the Markov Chain Monte Carlo algorithm), adjusting for all covariates. The 14 metals were grouped into three groups based on their common Spearman correlations with the same food group sources (Group 1: Al, Cd, Mn, Pb, Co, Ni, Sn, correlated with fruits; Group 2: As, CrVI, Ge, Li, Sb, V, correlated with water; Group 3: HgI, correlated with fish) to fit the hierarchical BKMR model (Fig. S2).

- Quantile g-computation (QGC) was finally performed to estimate the joint effect of the metal mixture, providing estimates of the simultaneous effect on the child neurodevelopment outcome when all exposures in the mixture increase by one quantile. The QGC model also displayed assigned weights of individual metals in the mixture, indicating the contribution of individual metals in the mixture to the joint effect. QGC is a novel method based on weighted quantile sum (WQS) to analyze mixtures, without requiring the assumption of directional homogeneity, and allow nonlinearity and non-additivity effects of the individual in mixture [50]. The QGC model also included 14 metals and adjusted for all covariates, employing 1000 bootstrap iterations for robustness.

All the statistical analyses were performed in R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria).

2.8 Ethical statement

The ELFE study received approvals from the National Advisory Committee on Information Processing in Health Research (Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé), the National Data Protection Authority (Comission Nationale Informatique et Liberté), the Committee for Protection of Persons Engaged in Research (Comité de Protection des Personnes) and the National Committee for Statistical Information (Conseil National de l'Information Statistique). An informed consent was signed by the parents or the mother alone, with the father being informed of his right to deny the consent for participation.

Results

3.1 Sample characteristics

Compared to excluded mothers, included mothers were older, more frequently employed, more highly educated, had higher household incomes, smoked less during pregnancy, and were less likely to be immigrants. Included and excluded children showed no difference in sex, but included children had a higher proportion of having only one sibling (Table 1).

Table 1 General characteristics of the included and excluded study population at baseline

Characteristics	Missing Value	Included population (N = 10,080)	Missing Value	Excluded population (N = 8,249)	p-value
Maternal age at delivery (years), median ± SD	1	31.5 ± 4.6	112	30.0 ± 5.5	**<0.001**
Maternal pre-pregnancy BMI (kg/m2), median ± SD	101	23.3 ± 4.6	328	23.7 ± 5.0	**<0.001**
Maternal educational level, % (n)	2,658		3,138		**<0.001**
Up to upper secondary		2.8% (210)		8.1% (412)	
Intermediate		36.7% (2,720)		54.2% (2,772)	
3-y university degree		33.1% (2,455)		22.3% (1,138)	
At least 5-y university degree		27.5% (2,037)		15.4% (789)	
Maternal employment status during pregnancy, % (n)	134		1,792		**<0.001**
Employed		78.5% (7,804)		60.5% (3,909)	
Unemployed		10.0% (998)		15.4% (997)	
Out of the labor force		11.5% (1,144)		24.0% (1,551)	
Household income, (€/month/consumption unit), median ± SD	405	1740 ± 929	2,385	1458 ± 1107	**<0.001**
Migration status, % (n)	154		1,768		**<0.001**
Immigrant		7.3% (726)		14.7% (954)	
Descendant of at least one immigrant		9.3% (923)		12.3% (799)	
Rest of population		83.4% (8,277)		73.0% (4,728)	
Maternal smoking during pregnancy, % (n)	93		262		**<0.001**
Never smoker		58.0% (5,795)		56.1% (4,479)	
Smoker only before pregnancy		25.3% (2,529)		19.4% (1,547)	
Smoker only in early pregnancy		3.6% (362)		4.4% (355)	
Smoker throughout pregnancy		13.0% (1,301)		20.1% (1,606)	
Child sex, % (n)	0		99		0.233
Boys		51.0% (5,141)		51.9% (4,230)	
Girls		49.0% (4,939)		48.1% (3,920)	
Number of older children, % (n)	127		1,659		**<0.001**
No sibling		43.1% (4,291)		46.7% (3,076)	
One sibling		38.9% (3,871)		32.8% (2,158)	
At least 2 siblings		18.0% (1,791)		20.6% (1,356)	

3.2 Concentration of metals

Table 2 displays the maternal dietary exposure to metals considered individually among the entire study sample, measured in units of µg/day, along with different types of health-based guidance values from the TDS 2 study. All the concentrations are reported using the median and IQR, as none exhibited a normal distribution.

Table 2 Concentrations of metals in the study sample and health-based guidance values in the TDS 2 study (n=10,080)

Chemicals	Concentration (µg/day), Median (IQR)	Health-based guidance values	Type of value adopted
Aluminum	3071.6 (2370.2-3354.6)	1000 µg/kg bw/week	PTWI
Arsenic (inorganic)	27.3 (21.1-35.3)	0.3-8 µg/kg bw/day	Reference point
Cadmium	11.4 (9.0-14.5)	2.5 µg/kg bw/week	TWI
Mercury (inorganic)	1.0 (0.7-1.3)	4 µg/kg bw/week	PTWI
Manganese	2278.6 (1762.8-2884.7)	10000 µg/day	UL
Lead	14.0 (11.3-17.4)	0.5-1.5 µg/kg bw/day	Reference doses
Cobalt	14.0 (11.2-17.5)	1.6-8 µg/kg bw/day	TDI
Chromium (VI)	49.4 (40.5-61.4)	/	/
Germanium	5.1 (3.7-6.9)	1000 µg/kg bw/day	LOAEL
Lithium	47.1 (35.1-61.8)	/	/
Nickel	171.5 (136.1-215.5)	22 µg/kg bw/day	TDI
Antimony	1.8 (1.4-2.4)	6 µg/kg bw/day	TDI
Tin	91.2 (60.3-141.2)	/	/
Vanadium	67.2 (53.3-85.8)	/	/

Reference point and reference doses are from The European Food Safety Authority (EFSA 2010b). Abbreviations: PTWI, provisional tolerable weekly intake; TWI, tolerable weekly intake; UL, tolerable upper intake level; TDI, tolerable daily intake; LOAEL, lowest observed adverse effect level; bw, body weight; IQR, interquartile range.

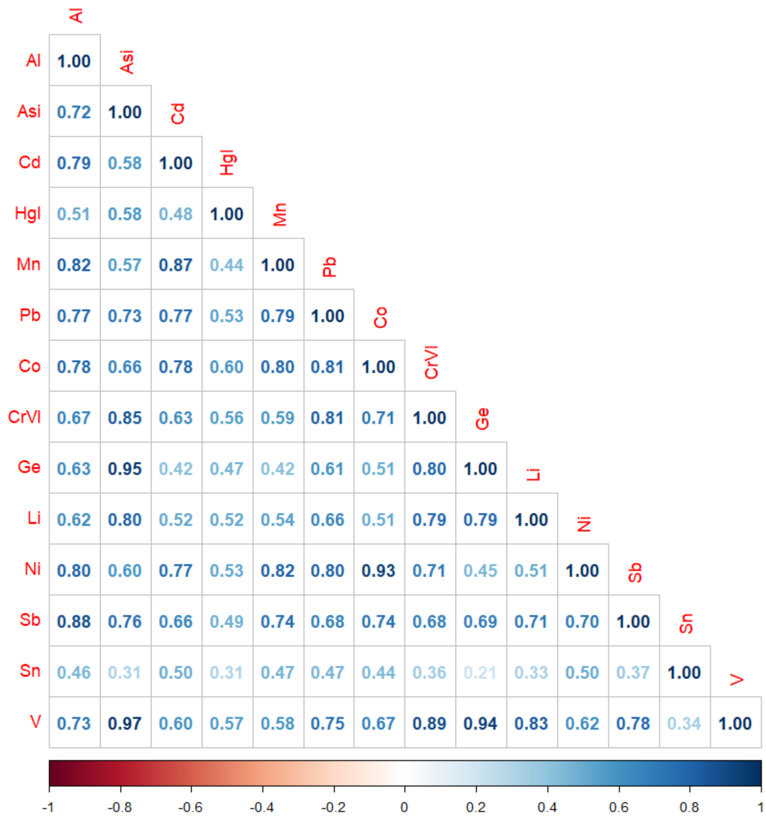
3.3 Correlation of dietary exposure to metals

A Spearman correlation matrix was conducted between each individual metals (Fig. 2), illustrating some metals were highly correlated.

3.4 Multivariable linear regression

The multivariable linear regression considered all 14 metals simultaneously and adjusted for potential confounding factors as previously described. Prenatal exposure to mercury, manganese, germanium and tin was positively associated with CDI-3.5 score, while prenatal dietary exposure to antimony was negatively associated with CDI-3.5 score (Table 3).

Fig. 2 Spearman correlation matrix of 14 metals (N=10,080)



Abbreviations: Al, aluminum; Asi, inorganic arsenic; Cd, cadmium; HgI, inorganic mercury; Mn, manganese; Pb, lead; Co, cobalt; CrVI, chromium (VI); Ge, germanium; Li, lithium; Ni, nickel; Sb, antimony; Sn, tin; V, vanadium.

Table 3 Associations of 14 metals considered simultaneously and CDI score at 3.5 years

Chemicals	**Adjusted model (N= 10,080)**	
	β (95%CI)	**p-value**
Aluminum	0.59 (-0.31, 1.49)	0.196
Arsenic (inorganic)	-1.58 (-3.26, 0.10)	0.066
Cadmium	-0.31 (-1.03, 0.41)	0.394
Mercury (inorganic)	**0.43 (0.13, 0.72)**	**0.004**
Manganese	**0.84 (0.03, 1.65)**	**0.043**
Lead	0.06 (-0.78, 0.91)	0.882
Cobalt	-0.17 (-1.33, 0.99)	0.770
Chromium (VI)	0.18 (-0.88, 1.23)	0.745
Germanium	**1.62 (0.53, 2.70)**	**0.004**
Lithium	0.24 (-0.40, 0.89)	0.464
Nickel	0.45 (-0.62, 1.52)	0.408
Antimony	**-1.17 (-2.03, -0.31)**	**0.008**
Tin	**0.34 (0.17, 0.52)**	**<0.001**
Vanadium	-0.55 (-2.18, 1.07)	0.505

Values were estimated (95%CI) from a multivariable linear regression model, considering all metals simultaneously and also adjusted for maternal age at delivery, maternal pre-pregnancy body mass index, maternal educational level, maternal employment status during pregnancy, household income, Edinburgh Postnatal Depression Scale, migration status, number of older children, maternal smoking during pregnancy, maternal daily energy intake, maternal diet quality, region of residence, recruitment wave and maternity size. Abbreviations: CI, confidence interval.

3.5 Principal component analysis

Based on the principal component analyses, 4 patterns of dietary exposure to metals were identified in PCA, which collectively explained 89% of the variance (Table 4). Factor loadings greater than 0.40 were considered indicative of a strong contribution to the underlying pattern. The first pattern, labeled 'global exposure to metals', was positively associated with all the metals. The second pattern, labeled 'High Mn, Sn and low Ge', was positively associated with Mn and Sn, and negatively associated with Ge. The third pattern, labeled 'Sn', was characterized by high dietary exposure to Sn. The final pattern, labeled 'HgI', was characterized by high dietary exposure to HgI.

Table 4 Factor loadings of dietary exposure patterns to 14 metals (n=10,080)

Chemicals	**Components**			
	1	2	3	4
Aluminum	**0.89**	0.17	-0.15	-0.11
Arsenic (inorganic)	**0.89**	-0.39	0.01	-0.02
Cadmium	**0.82**	0.38	-0.09	-0.05
Mercury (inorganic)	**0.65**	-0.05	0.28	**0.66**
Manganese	**0.83**	**0.41**	-0.18	-0.09
Lead	**0.89**	0.14	-0.02	0.01
Cobalt	**0.87**	0.31	-0.12	0.19
Chromium (VI)	**0.88**	-0.24	0.05	0.03
Germanium	**0.78**	**-0.57**	0.00	-0.10
Lithium	**0.80**	-0.38	0.11	-0.12
Nickel	**0.85**	0.37	-0.08	0.10
Antimony	**0.87**	-0.03	-0.17	-0.15
Tin	**0.51**	**0.40**	**0.70**	-0.29
Vanadium	**0.90**	-0.38	0.02	-0.05
% Explained variance	68	11	5	5
Cumulative % Explained variance	68	79	84	89

A multivariable linear regression model was performed considering all 4 PCA components simultaneously instead of 14 individual metals, and adjusted for potential confounding factors. All 4 dietary exposure patterns were positively associated with CDI score at 3.5 years. (Table 5).

Table 5 Associations between 4 exposure patterns to metals and CDI score at 3.5 years (n=10,080)

Components	β (95%CI)	p-value
1	**2.02 (1.82, 2.22)**	**<0.001**
2	**0.16 (0.09, 023)**	**<0.001**
3	**0.11 (0.003, 0.21)**	**0.042**
4	**0.23 (0.10, 0.36)**	**<0.001**

Values are estimated (95%CI) from a multivariable linear regression model, considering all 4 PCA components simultaneously and also adjusted for maternal age at delivery, maternal pre-pregnancy body mass index, maternal educational level, maternal employment status during pregnancy, household income, Edinburgh Postnatal Depression Scale, migration status, number of older children, maternal smoking during pregnancy, maternal daily energy intake, maternal diet quality during the last trimester of pregnancy, region of residence, recruitment wave and maternity size.
Abbreviations: CI, confidence interval.

3.6 Bayesian kernel machine regression

The group conditional posterior inclusion probabilities (groupPIPs) and conditional posterior inclusion probabilities (condPIPs) derived from the BKMR model are presented in Table 6. The posterior inclusion probability (PIP) is a ranking measure (ranging from 0 to 1) used to indicate the extent to which data supports the inclusion of variables. In BKMR models, groupPIPs assessed the importance of three metal groups, and condPIPs assessed the importance of individual metals within their respective groups.

Group 1 had the highest contribution to the exposure-response function, with a groupPIP of 1.000. The groupPIPs for Group 2 and Group 3 were 0.516 and 0.518, respectively. In Group 1, Sn had the highest contribution with a condPIP of 0.970, while CrVi and Sb had the highest condPIPs in group 2 (0.674 and 0.112, respectively). HgI was the only metal in Group 3 with a condPIP 1.000.

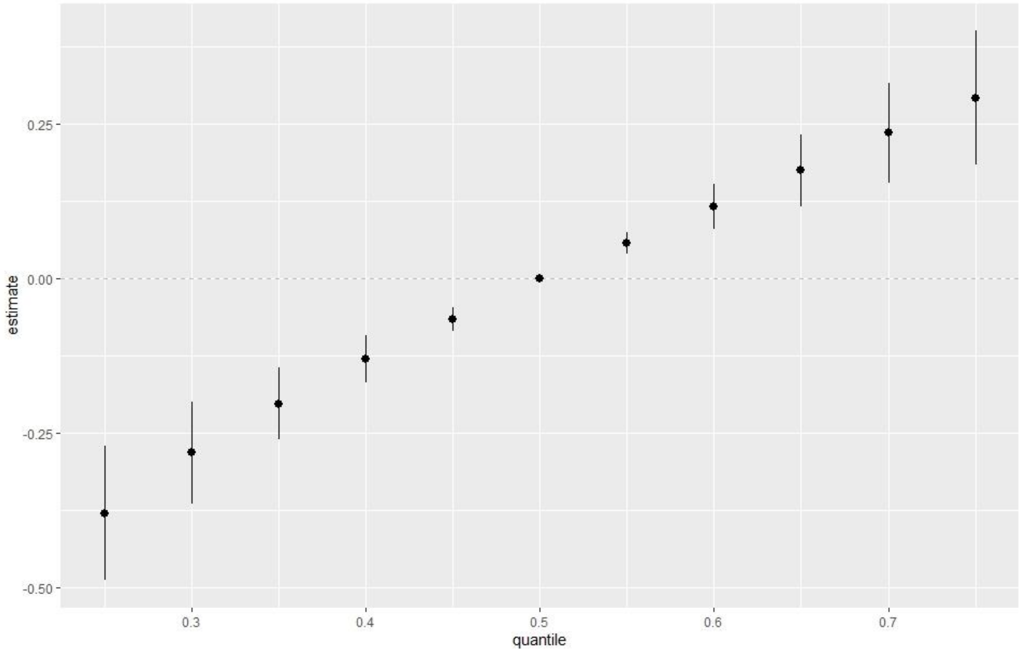
Table 6 Group and conditional posterior inclusion probabilities of the metal mixture in BKMR (n=10,080)

Chemicals	Group	GroupPIP	CondPIP
Aluminum	1	1.000	0.022
Cadmium	1	1.000	0.000
Manganese	1	1.000	0.000
Lead	1	1.000	0.000
Cobalt	1	1.000	0.000
Nickel	1	1.000	0.008
Tin	1	1.000	0.970
Arsenic (inorganic)	2	0.516	0.023
Chromium (VI)	2	0.516	0.674
Germanium	2	0.516	0.062
Lithium	2	0.516	0.054
Antimony	2	0.516	0.112
Vanadium	2	0.516	0.074
Mercury (inorganic)	3	0.518	1.000

The BKMR model included all metals simultaneously and adjusted for maternal age at delivery, maternal pre-pregnancy body mass index, maternal educational level, maternal employment status during pregnancy, household income, Edinburgh Postnatal Depression Scale, migration status, number of older children, maternal smoking during pregnancy, maternal daily energy intake, maternal diet quality, region of residence, recruitment wave and maternity size.
Abbreviations: GroupPIP, group posterior inclusion probability; CondPIP, conditional posterior inclusion probability.

In the overall mixture effect analysis, after adjustment for all the potential confounding factors, the log-transformed concentration of metals mixture at or over the 60th percentile was positively associated with the CDI-3.5 score (Fig. 3).

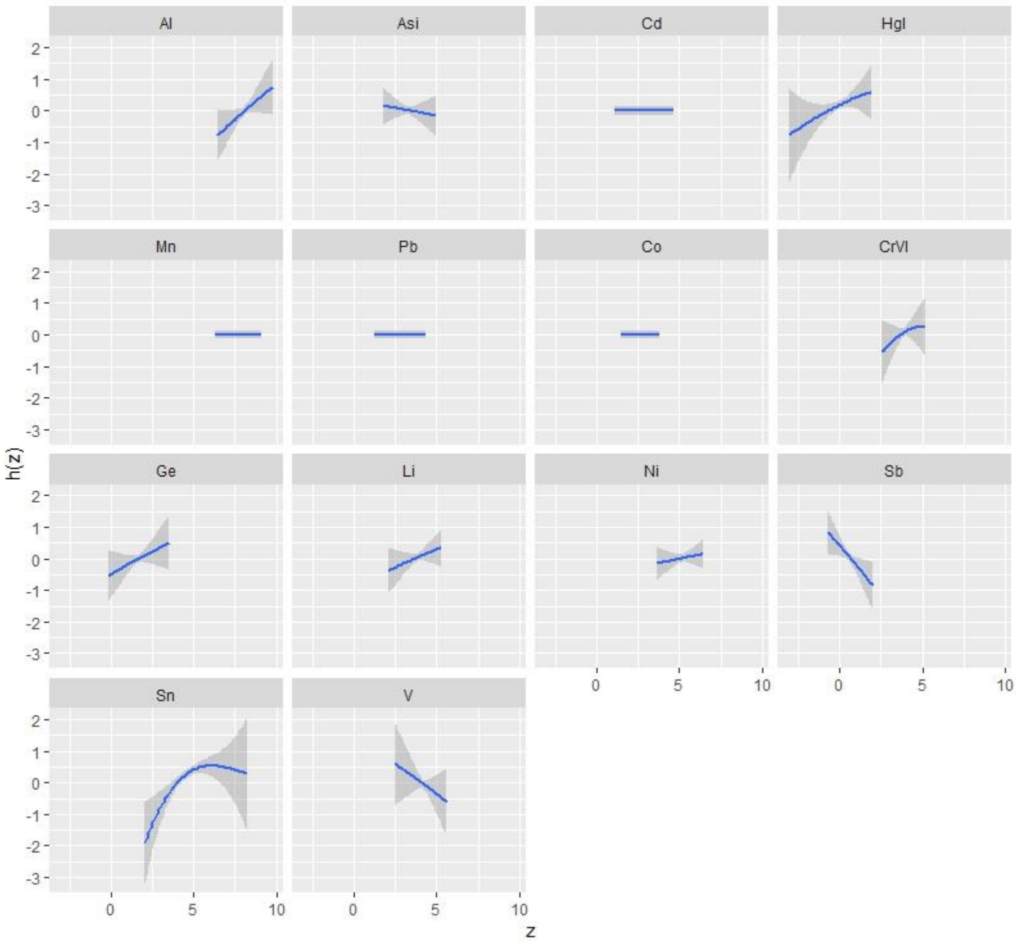
Fig. 3 Overall association of the metal mixture and the CDI-3.5 score in BKMR (n=10,080)



This figure plots the estimated difference in CDI-3.5 score when all metals were fixed at specified quantiles, ranging from the 25th percentile to the 75th percentile. The BKMR model included all metals simultaneously and adjusted for maternal age at delivery, maternal pre-pregnancy body mass index, maternal educational level, maternal employment status during pregnancy, household income, Edinburgh Postnatal Depression Scale, migration status, number of older children, maternal smoking during pregnancy, maternal daily energy intake, maternal diet quality, region of residence, recruitment wave and maternity size.

Next, we analyzed univariate associations to visually examine the individual effect of each metal in the mixture on child neurodevelopment outcome while holding the other metals constant at the 50th percentile (Fig. 4). The exposure-response function (h) curves indicated that HgI had a linear positive relationship, whereas Sn had a non-linear positive relationship, and Sb had a linear negative relationship with the outcome, all with credible intervals excluding the null at one point.

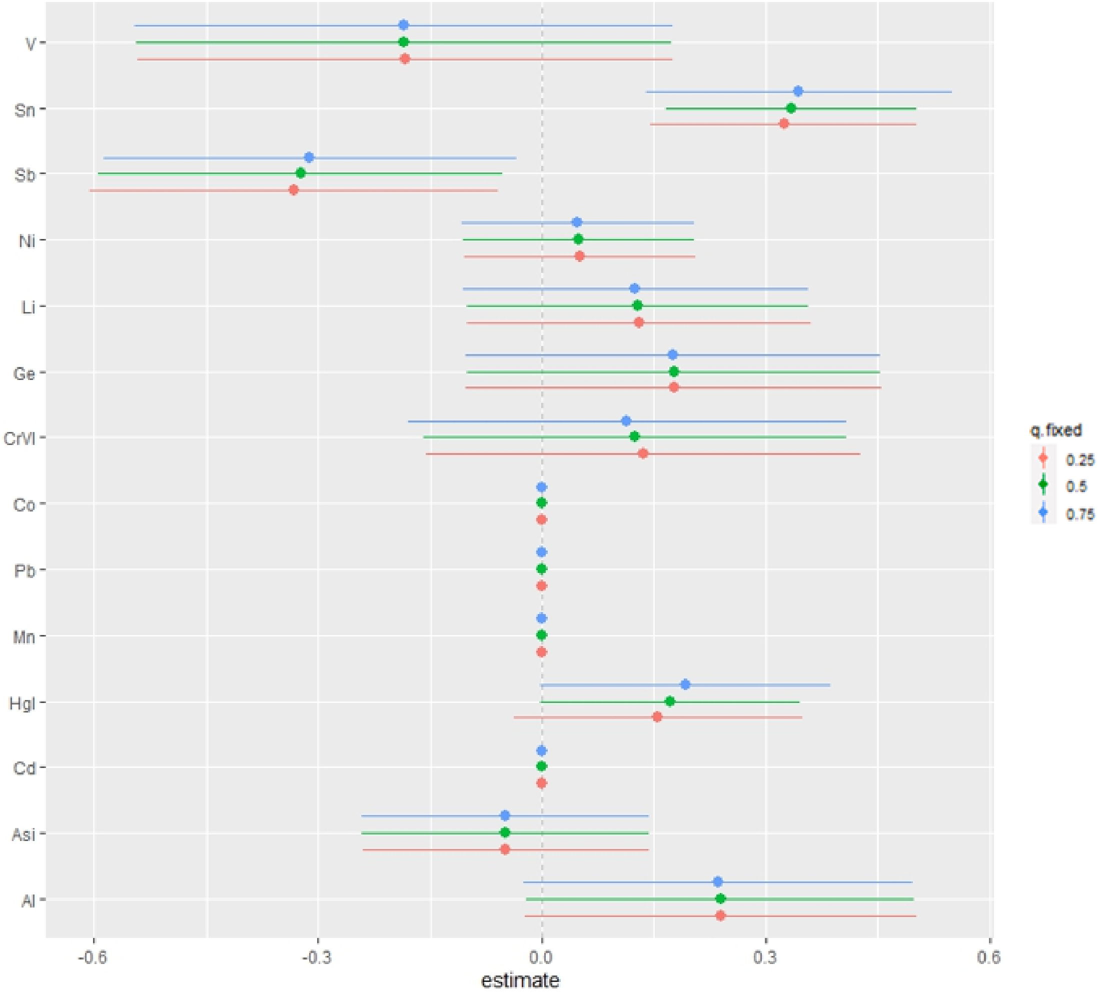
Fig. 4 Univariate associations of individual metals and the CDI-3.5 score in BKMR (n=10,080)



This figure shows the univariate associations of individual metals and CDI-3.5 score in BKMR, when all other metals were fixed at the 50th quantile. The BKMR model included all metals simultaneously and adjusted for maternal age at delivery, maternal pre-pregnancy body mass index, maternal educational level, maternal employment status during pregnancy, household income, Edinburgh Postnatal Depression Scale, migration status, number of older children, maternal smoking during pregnancy, maternal daily energy intake, maternal diet quality, region of residence, recruitment wave and maternity size. The predictors z are referred to as exposure variables, and h(z) is termed the exposure-response function.

Single-variable effects of individual metals are presented in Fig. 5. The individual contributions to the outcome were assessed when other metals were fixed at the 25th, 50^{th} and 75th percentile, respectively. The results were consistent with univariate associations analysis: prenatal exposure to Sn and HgI were positively associated, while prenatal dietary exposure to Sb was negatively associated with the outcome.

Fig. 5 Associations of individual metals and the CDI-3.5 score in BKMR (n=10,080)



This figure displays the estimated difference in the CDI-3.5 score with a change in individual metals, when all other metals were fixed at the 25th (red), 50th (green) and 75th (blue) percentile. The BKMR model included all metals simultaneously and adjusted for maternal age at delivery, maternal pre-pregnancy body mass index, maternal educational level, maternal employment status during pregnancy, household income, Edinburgh Postnatal Depression Scale, migration status, number of older children, maternal smoking during pregnancy, maternal daily energy intake, maternal diet quality, region of residence, recruitment wave and maternity size.

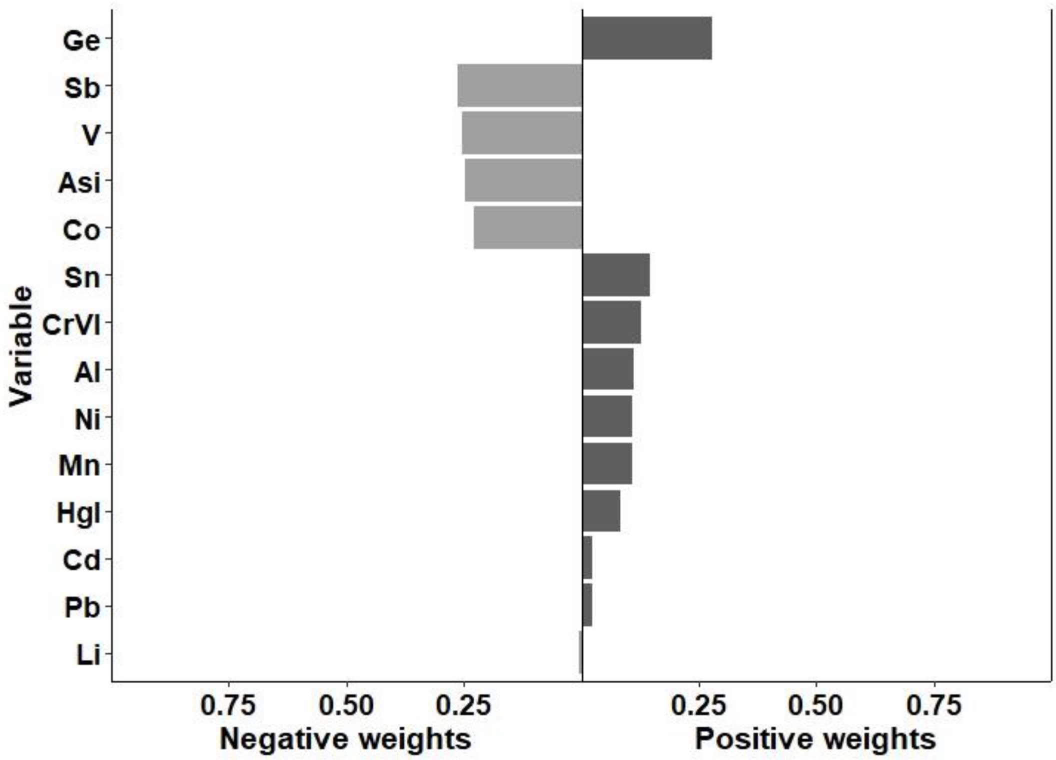
Fig. S3 illustrates the interaction analyses between each pair of metals. The bivariate exposure-response function curve of one metal is depicted, when the concentration of another metal in the pair was held constant at the 25th, 50th, or 75th percentile with all other metals simultaneously fixed at the 50th percentile, respectively. No interactions were detected within the 14 metals.

3.7 Quantile g-computation

The joint effect of the mixture from the results of QGC was consistent with the overall effect of BKMR model, indicating that for every one quantile increase in the log-transformed concentration of the metal mixture, there is a corresponding increase in the CDI-3.5 score (β=0.53, 95%CI: 0.29, 0.77).

The individual metals included in the mixture were related to the CDI-3.5 score in different directions, illustrated by the assigned weights (Fig. 6). Ge contributed the most to the outcome in the positive direction, followed by Sn, CrVI, Al, Ni, Mn and HgI. In the negative direction, Sb had the highest contribution to the outcome, followed by V, Asi and Co. Cd, Pb and Li only had marginal contributions to the outcome.

Fig. 6 The directions and magnitudes of the assigned weights for individual metals in relation to the CDI-3.5 score in the QGC model (n=10,080)



The model considered all metals simultaneously and adjusted for maternal age at delivery, maternal pre-pregnancy body mass index, maternal educational level, maternal employment status during pregnancy, household income, Edinburgh Postnatal Depression Scale, migration status, number of older children, maternal smoking during pregnancy, maternal daily energy intake, maternal diet quality, region of residence, recruitment wave and maternity size.

Discussion

Multiple statistical methods were applied in this study to investigate the effects of maternal dietary exposure to individual metals and metal mixture on child neurodevelopment outcome. Our main findings were as follows: (1) The overall effect of prenatal exposure to the 14 metals was positively associated with the CDI score at 3.5 years. (2) In both the multivariable linear regression model considering all metals simultaneously and the BKMR model, we observed that mercury and tin had positive associations with child neurodevelopment outcome, whereas antimony had a negative association. The directions of these associations were consistent with the assigned weights of individual metals in the QGC model. (3) In the multivariable linear regression model considering all metals simultaneously, we additionally observed that manganese and germanium had positive associations with the CDI-3.5 score. The directions of these associations were also consistent with the assigned weights in the QGC model. (4) In PCA, the first four principal components, explaining 89% of the variance, strongly represented key patterns of maternal dietary exposure to the 14 metals. We observed that in the multivariable linear regression model using the 4 components instead of 14 metals, the directions of the coefficients for these 4 components were consistent with the directions of the coefficients for the individual metals representing these components' characteristics in the linear regression model considering all metals simultaneously.

The results of the four methods were broadly consistent. Linear regression models, alongside logistic regression models, are the classical and most widely used approaches for studying chemical substances, yielding straightforward and interpretable results [51,52]. However, its application to study mixtures is particularly limited due to the prevalent interactions or collinearity among chemicals in real-world mixtures [53,54]. Linear regression struggles to handle such interactions or collinearity effectively in high-dimensional data.

There is insufficient evidence to suggest that germanium is neurotoxic, despite one animal study reporting germanium dioxide-induced neurotoxicity [55]. Through the Spearman correlation matrix, we observed that germanium was highly correlated with arsenic (r=0.95) and vanadium (r=0.94). Its contribution in the BKMR model was minimal, with a condPIP of 0.062. This indicates that the significant association of germanium in multivariable linear regression may be spurious.

Manganese is a fundamental element in numerous physiological processes, encompassing protein and energy metabolism, cellular defense against detrimental free radicals, bone development, immune response, reproductive health, digestion, and metabolic homeostasis [56]. Despite being a well-understood essential trace element, excessive manganese exposure can lead to neurotoxicity [57]. Literature shows mixed epidemiological findings about prenatal exposure to manganese and child neurodevelopment outcomes. One study using PROGRESS birth cohort reported a negative association between prenatal exposure to manganese and child neurodevelopment at 24 months of age (n=514) [58]; another cohort study in Mexico found no association between maternal blood manganese levels and child neurodevelopment in the first, third, sixth, and twelfth months (n=253) [59], and one study in Spain found that manganese levels in placenta were associated with a decrement in perceptual-performance skills in a dose-response manner but with better memory span and quantitative skills at the age of 4–5 years (n=302) [60]. In the present study, dietary exposure to manganese is far below the tolerable upper intake level (Table 2). Therefore, the exposure level is probably too low to induce deleterious effects on child neurodevelopment. Moreover, through the Spearman correlation matrix, manganese was correlated with some other metals, potentially distorting the results.

Mercury has long been established as having neurotoxic effects, with seafood being the main source of mercury intake. However, literature shows mixed associations between prenatal exposure to mercury and neurodevelopment. We observed a positive association between mercury and child neurodevelopment, consistent with one article that reported maternal total mercury levels in biomarkers were positively associated with language composite score and receptive communication scaled score at 18 months of age (n=1,308) [61]. Two cohort studies found mercury exposure through fish intake was not associated with neurodevelopmental performance [62,63]. One cohort study in Korea reported maternal exposure to mercury during late pregnancy was negatively associated with the Mental Development Index score and Physical Development Index score at 6 months in infants (n=523) [64]. A possible explanation is that n-3 polyunsaturated fatty acids (PUFAs) in fish consumption have significant health benefits for humans [65]. Moreover, the dietary exposure to mercury in the present study was much lower than the provisional tolerable weekly intake (Table 2). However, different speciation of mercury, such as total mercury and methylmercury, were used in the literature, which the present study did not include in the analysis because they were highly correlated with fish consumption (>0.8).

Tin is classified as potentially toxic by the WHO. Inorganic tin has low toxicity, whereas many organic tin compounds are toxic [66]. Although we observed positive association of tin with child neurodevelopment, the health benefits of tin for humans remain unknown. One prospective study in China found urinary Tin levels were negatively associated with Intelligence Quotient (IQ) at ages 8 and 10 [67]. No literature or health-based guidance values were available to compare the dietary exposure level to tin in the present study.

Among the four statistical models, the only metal negatively associated with child neurodevelopment outcome was antimony. Antimony and its compounds occur naturally in the Earth's crust and are released into the environment through natural processes [68]. The various toxic effects of antimony on the human body have been confirmed in occupational exposure among process workers and laboratory animals [68–70]. Two prospective studies in China showed prenatal exposure to antimony in mixtures was negatively associated with

Gesell Development Scale at 2-3 years of age and IQ at 7-10 years of age, respectively [71,72], which are consistent with our results.

The expected negative associations between metals documented to have neurotoxic effects on neurodevelopment, such as lead and arsenic, were not observed in the present study. Several possible explanations could help to comprehend our results: (1) Although the concentrations of these 14 metals are not comparable to those in most previous studies, because most literature focuses on substance levels in biomarkers. However, according to Table 2, it is possible that because the concentrations of these 14 metals are far below the health-based guidance values in TDS2, they may not have had adverse effects on neurodevelopment yet. (2) The rate at which chemicals pass through the placenta varies, leading to different levels of accumulative chemicals in infant circulation. For example, one study in Japan found that antimony levels in the cord blood were twofold higher than those in the maternal blood [73]. A population study in China reported that lead, manganese, nickel, chromium, tin, vanadium, and arsenic could be detected in umbilical cord blood, while cadmium showed difficulty in crossing the placental barrier [74]. Therefore, maternal dietary exposure during pregnancy may not accurately represent fetal exposure to some chemicals.

Our study had several strengths. Firstly, it is the first prospective study investigating the relationship between maternal dietary exposure to metal mixture and child neurodevelopment in a large-scale population-based cohort, addressing a gap in previous research. Secondly, we applied various statistical methods to analyze the effects of the overall mixture and individual metals in the mixture, and the different methods resulted in broadly consistent findings. Lastly, we adjusted models for various potential confounders selected using a DAG.

There are also some limitations in our study. Firstly, FFQ might reduce accuracy due to recall bias [75]. The FFQ used in the study of dietary intake has been previously validated to mitigate this bias [76]. Secondly, the differences in characteristics between included and excluded populations at baseline might result in some degree of selection bias. The ELFE study provides an opportunity to address this selection and attrition bias, using a specific calculated weighting. However, due to time constraints during the internship, we were unable to address this issue. This will be undertaken in the future. Other studies on similar subjects utilizing the ELFE study demonstrated that this attrition bias minimally impacted our findings [77]. Lastly, We excluded some chemicals highly correlated with specific food items, such as methylmercury highly correlated with fish consumption (r=0.9), potentially limiting the comprehensiveness of the results. However, this exclusion aimed at reducing potential confounding effects from specific dietary sources. It ensured that observed associations between chemical exposures and child neurodevelopmental outcomes were more likely attributable to a broader range of chemical exposures rather than solely to specific dietary components. Therefore, the implications of this exclusion warrant further consideration.

Conclusion

The present study applied multivariable linear regression, PCA, BKMR and QGC models to investigate the associations between maternal dietary exposure to food chemical mixture of 14 metals and child neurodevelopment. In the context of prenatal co-exposure to 14 metals, we obtained consistent results across all four statistical methods. The overall effect of the metal mixture was positively associated with child neurodevelopment at 3.5 years of age. Specifically, antimony exhibited a negative association, whereas inorganic mercury and tin showed positive associations with CDI-3.5 score. Additionally, manganese and germanium demonstrated positive associations only in the multivariable linear regression. No associations were found with other metals.

Our study suggests that given the complexity of chemical mixtures, it is crucial to apply different statistical methods to assess their impact on health. We recommend integrating findings from various approaches to derive more reliable conclusions. Regarding the recommendations that can be made to the public based on this study, we must exercise caution, as this is the first large-scale population-based longitudinal study on the association of prenatal exposure to food chemicals and child neurodevelopment. Despite identifying several associations, such as soda being the food most highly associated with antimony, we cannot yet provide dietary recommendations from a public health perspective due to the complexity of various limitations and issues. This study represents just the initial phase in this research direction. The exact mechanism of the association between prenatal exposure to metals and child neurodevelopment is still unclear, and requires further investigation in future studies.

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Appendices

Fig. S1 Directed acyclic graph

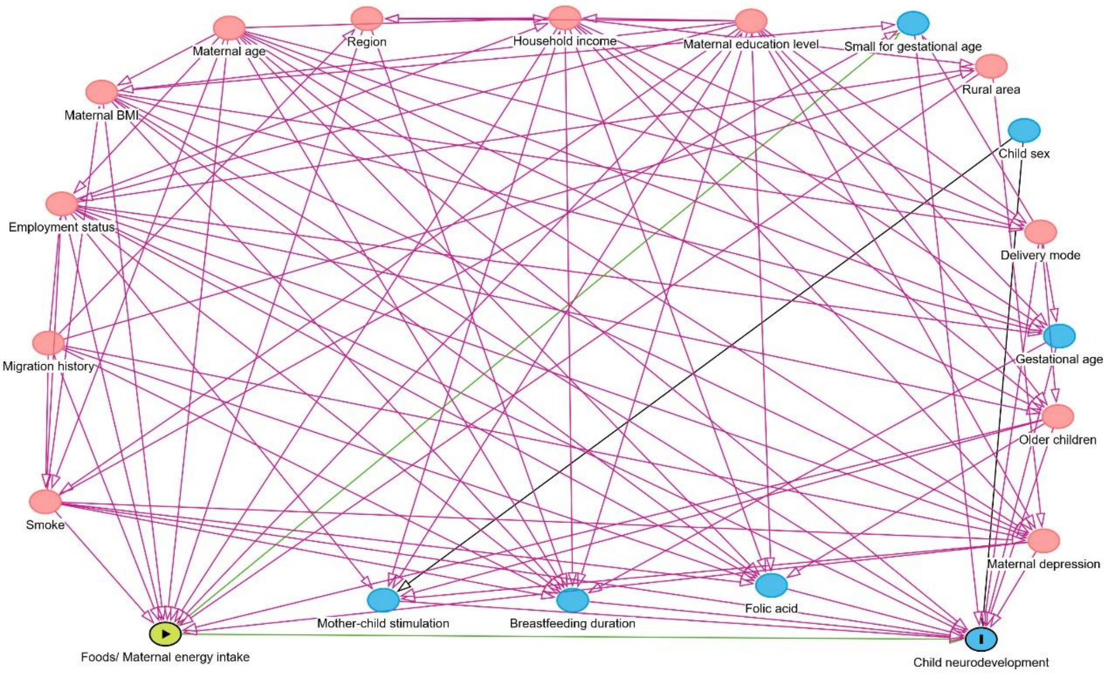
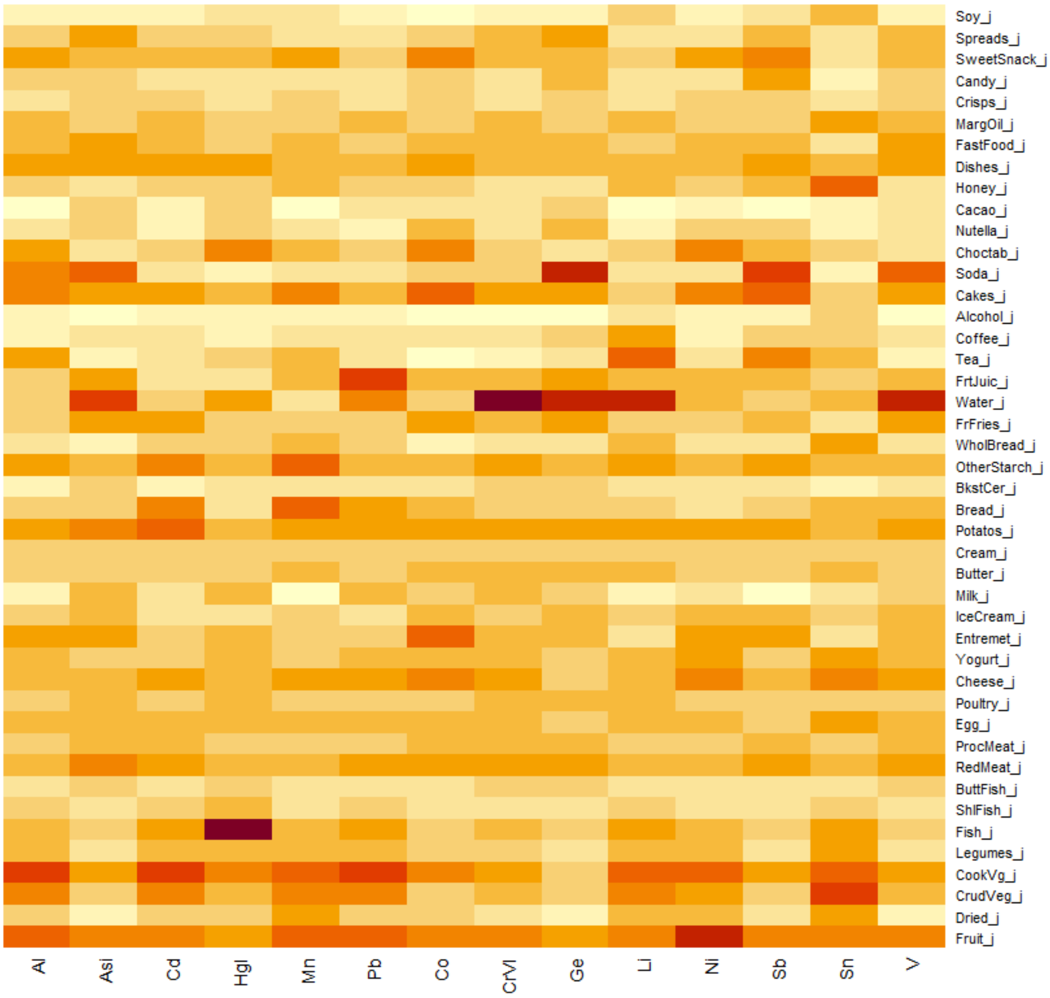
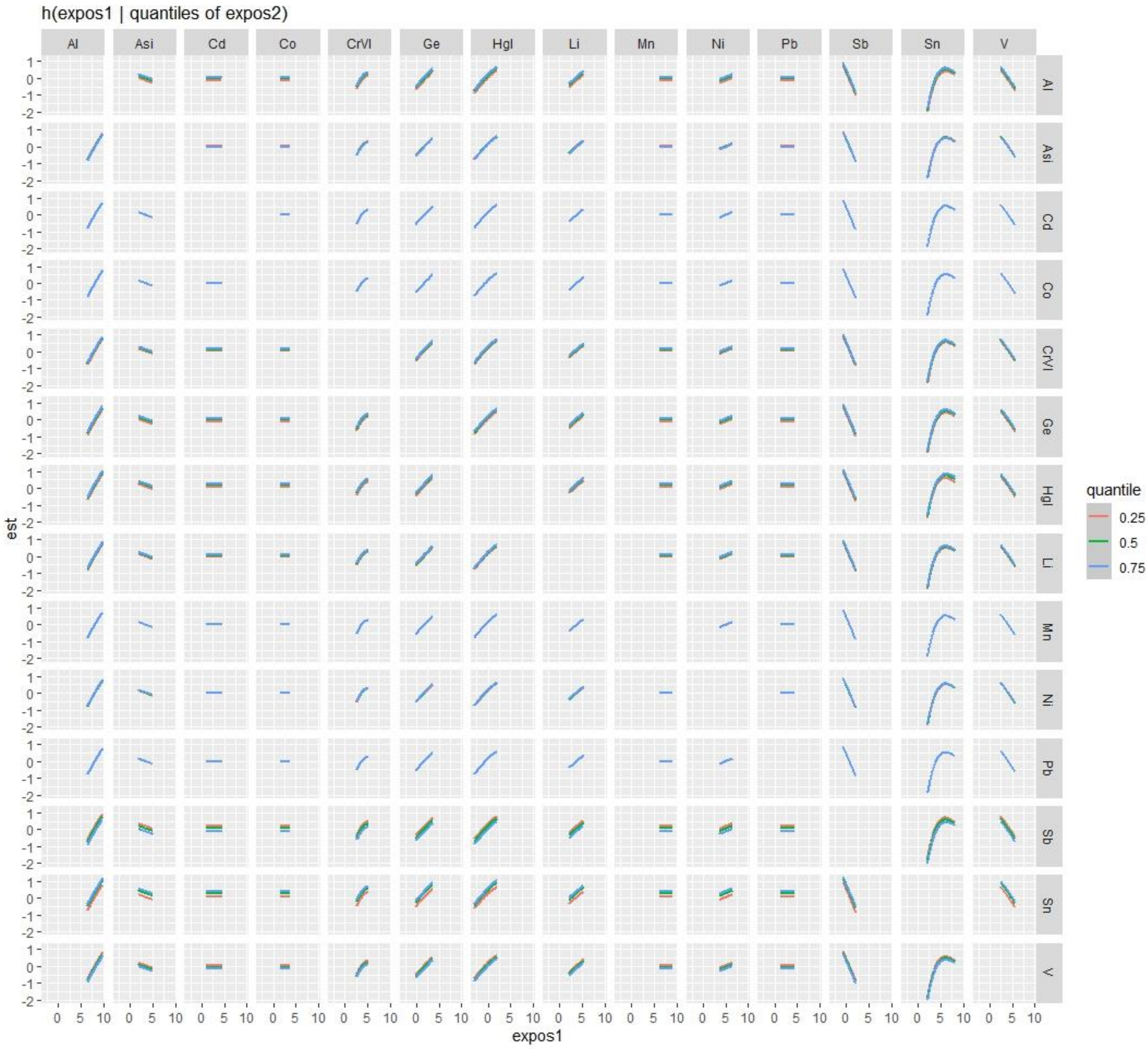


Fig. S2 Spearman correlation heatmap of 14 metals and 44 food groups



Darker colors indicate stronger correlations

Fig. S3 Bivariate exposure-response relationships between each pair of individual metals



This figure shows bivariate exposure-response relationships between each pair of individual metals, while the concentration of another individual metal was held constant at the 25th (red), 50th (green) and 75th (blue) percentile with all other metals simultaneously fixed at the 50th percentile, respectively. The BKMR model included all metals simultaneously and adjusted for maternal age at delivery, maternal pre-pregnancy body mass index, maternal educational level, maternal employed during pregnancy, household income, Edinburgh Postnatal Depression Scale, migration status, number of older children, maternal smoking during pregnancy, maternal daily energy intake, maternal diet quality during the last trimester of pregnancy, region of residence of the family, recruitment wave and maternity size.

Résumé

Contexte : La première enfance est une période critique pour le neurodéveloppement, durant laquelle le système nerveux est hautement vulnérable aux facteurs environnementaux. L'exposition maternelle aux produits chimiques pendant la grossesse peut se transférer à travers le placenta vers la circulation fœtale. De nombreuses substances ont été documentées pour leur neurotoxicité avérée ou potentielle. L'alimentation constitue l'une des voies principales d'exposition à ces produits chimiques. L'objectif de la présente étude est d'investiguer les associations entre l'exposition alimentaire maternelle aux produits chimiques alimentaires au cours des trois derniers mois de la grossesse et le neurodéveloppement de l'enfant.

Méthodes : Un total de 10 080 dyades mère-enfant issues de l'étude Étude Longitudinale Française depuis l'Enfance (ELFE) ont été incluses dans la présente étude. L'exposition alimentaire maternelle aux produits chimiques alimentaires a été évaluée à l'aide d'un questionnaire de fréquence alimentaire (QFA). Le neurodéveloppement de l'enfant a été évalué à l'aide du score de l'Inventaire du Développement de l'Enfant (IDE) à l'âge de 3,5 ans. Après le dépistage de 210 produits chimiques, nous avons concentré notre analyse sur 14 métaux sous forme de mélange. Nous avons utilisé plusieurs méthodes statistiques pour analyser les effets de ces produits chimiques. L'analyse en composantes principales (PCA) a été utilisée pour réduire la dimensionnalité et appliquée à la régression linéaire multivariable. La régression bayésienne par noyaux (BKMR) et la g-computation quantile (QGC) ont été utilisées pour évaluer les multiples expositions aux mélanges.

Résultats : Nous avons obtenu des résultats cohérents avec les quatre méthodes statistiques utilisées. L'effet global du mélange de métaux était positivement associé au neurodéveloppement de l'enfant à l'âge de 3,5 ans. En particulier, l'Antimoine a montré une association négative, tandis que le Mercure inorganique et l'Étain ont montré des associations positives avec le neurodéveloppement de l'enfant. De plus, le Manganèse et le Germanium ont montré des associations positives dans la régression linéaire. Aucune association n'a été trouvée avec les autres métaux.

Conclusion : La présente étude a mis en évidence diverses associations entre l'exposition alimentaire maternelle aux métaux pendant la grossesse et le neurodéveloppement de l'enfant.